In re Application of
GREENBERGER, LEE MARTIN

Application No. 10/666,722
Filed: September 18, 2003

Art Unit 1614
Examiner Timothy Betton

METHOD OF TREATING RESISTANT TUMORS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

A F FI D A V I T UNDER 37 C.F.R. SECTION 1.132

STATE OF NEW YORK)
COUNTY OF ROCKLAND)

Frank Loganzo, Jr. residing at 7 Gregory Street, New City, NY, being duly sworn deposes and says:

THAT he is trained in Biochemistry & Molecular Biology, having received a Ph.D. in 1992, followed by successive post-doctoral training at Sloan-Kettering and Glaxo Wellcome for a total of 4 years.

THAT he has been employed as a senior research scientist at Wyeth for 11.5 years and has led multiple project teams, generating and reviewing data such as the type referred to in Exhibit 1 attached to this affidavit.

THAT his current title is Principal Research Scientist II, Discovery Oncology, Wyeth Research, Pearl River, New York.

THAT he has read and is familiar with the above-identified application for United States Letters Patent and with the Office Action thereto, mailed December 14, 2007.

THAT at his direction and under his supervision, or with his direct knowledge, in vivo animal studies were conducted in mouse models, as exemplified in the studies described in Exhibit 1 hereto.

THAT the results described in Exhibit 1 show that tumors are effectively treated by the Example 57 compound. Tumor growth is inhibited and/or eradicated at several dosage levels.

Further deponent sayeth not.

Frank Loganzo, Jr., Ph.D.

Sworn to and subscribed before me this 11th day of April, 2008.

NOTARY PUBLIC

JANICE A. WHITE
Notary Public - State of New York
No. 01-WH6052604
Qualified in Rockland County
My Commission Expires 12-26-2010

Exhibit 1

Example 57 inhibits the growth of a human tumor cell line grown in a mouse model of human cancer

Example 57 effectively inhibits the growth of the human melanoma cell line, Lox, when grown as a subcutaneous tumor in athymic mice (Table 1). Tumors were allowed to growth to a size of ~100 mg and then mice were administered various doses of the test agents on days 1, 5, and 9. By day 14, the Example 57 compound inhibited growth by 99% compared to vehicle control following 0.3 mg/kg and greater doses. Example 57 was as effective as Example 129 (a compound that underwent clinical phase I and II testing in humans) and paclitaxel (a standard chemotherapy currently approved for use in humans with cancer). In addition, "cures" were observed upon treatment of the Example 57 compound in this tumor model (refer to 0.6 mg mg/kg dose, showing relative tumor growth of "0" on day 21 and beyond). These effects were statistically significant and are representative of at least five other studies using this tumor model.

Table 1: Dose-response comparison of Example 57, Example 129, and paclitaxel with respect to growth effect of Lox human melanoma xenograft as subcutaneous tumors in athymic mice.

		Day 7			Day 14			Day 21	Day 28	Day 34
Example	Dose (mg/kg)	RTG	% T/C	*P-Value*	RTG	% T/C	*P-Value*	RTG	RTG	RTG
Saline	-	**11.7**			**34.0**					
57	1.6	**1.30**	11	*<0.01*	**0.21**	1	*<0.01*	**0.00**	**0.02**	**1.18**
57	1.5	**0.94**	8	*<0.01*	**0.20**	1	*<0.01*	**0.00**	**1.42**	**6.83**
57	1.4	**0.76**	7	*<0.01*	**0.22**	1	*<0.01*	**0.00**	**0.28**	**1.84**
57	0.6	**0.54**	5	*<0.01*	**0.06**	1	*<0.01*	**0.00**	**0.00**	**0.00**
57	0.5	**0.71**	6	*<0.01*	**0.30**	1	*<0.01*	**0.07**	**0.08**	**1.99**
57	0.3	**1.49**	13	*<0.01*	**0.22**	1	*<0.01*	**0.51**	**5.23**	**9.57**
57	0.2	**1.87**	16	*<0.01*	**1.63**	5	*0.01*	**14.0**	**26.3**	-
57	0.1	**2.95**	25	*<0.01*	**4.79**	14	*0.05*	**28.5**	-	-
129	0.3	**0.71**	6	*<0.01*	**0.23**	1	*<0.01*	**0.56**	**4.53**	**12.5**
Paclitaxel	60	**0.66**	6	*<0.01*	**0.09**	1	*<0.01*	**0.00**	**0.00**	**0.00**

Treatments on days 1, 5, 9 by intravenous (IV) administration. Measurements made on indicated days 7, 14, 21, 28, 34. Survival was 100% for all mice at the indicated doses during the study. RTG = relative tumor growth compared to day 0; %T/C = relative tumor growth of treated vs. control (vehicle) group; P-value = Student's t-test significance.

Example 57 inhibits the growth of tumors derived from different human cancers in a mouse model of human cancer

Example 57 was tested in several tumor models of human cancer in xenograft efficacy studies. Example 57 effectively inhibits the growth of human tumor cell lines derived from colon carcinoma, breast carcinoma, and melanoma (Table 2). Note that the activity of Example 57 is approximately equivalent to that of the clinically-used chemotherapeutic agents paclitaxel or vincristine at inhibiting growth of the Lox and MX1 cell lines, respectively. Compare day 14 values of 1% vs 1% T/C values for Lox and 0% vs. 6% T/C values for MX1. Example 57 shows slightly improved activity against HT29 compared with vincristine by day 7 (54% vs. 73% at day 7). Moreover, Example 57 is significantly more effective than vincristine at inhibiting the DLD1 colon cancer cell line (compare day 14 values of 5% vs. 77% T/C). These data suggest that Example 57 effectively inhibits growth of human cancers in a mammalian model of human cancer, and is equivalent or improved over existing anti-cancer agents of similar class.

Table 2: Effect of Example 57 and reference inhibitors on the growth of human xenograft tumors in athymic mice.

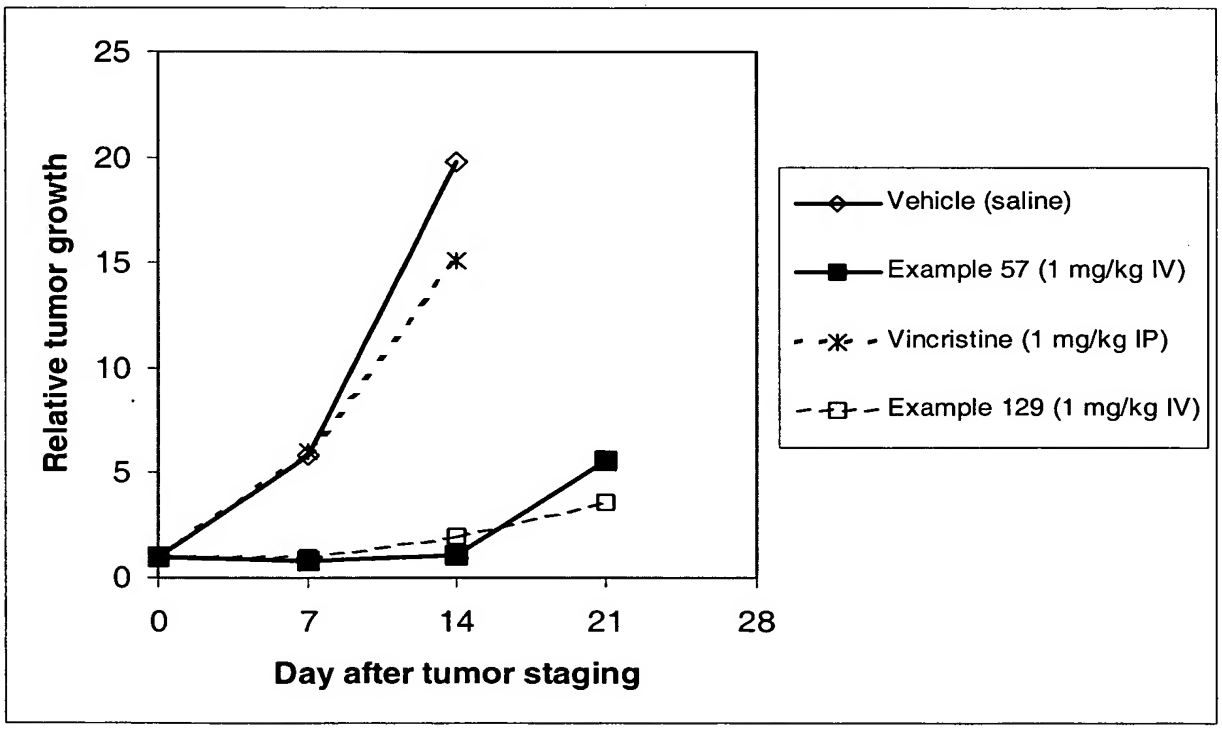
		Day 7			Day 14			Day 18-23	Day 25-30	Day 34-35
Example	Dose (mg/kg)	RTG	% T/C	*P-Value*	RTG	% T/C	*P-Value*	RTG	RTG	RTG
Tumor: Lox melanoma										
57	1	**0.62**	7	*<0.01*	**0.21**	1	*<0.01*	**0**	**0.23**	**6.2**
Paclitaxel	60	**0.41**	4	*<0.01*	**0.1**	1	*<0.01*	**0**	**0**	**0**
Tumor: DLD1 colon carcinoma										
57	1	**0.83**	14	*<0.01*	**1.1**	5	*<0.01*	**5.6**	-	-
Vincristine	1	**6.0**	103	*0.62*	**15.1**	77	*0.37*	-	-	-
Tumor: MX1 breast carcinoma										
57	1	**0.6**	18	*<0.01*	**0**	0	*<0.01*	**0**	**0**	-
Vincristine	0.8	**0.64**	19	*<0.01*	**0.69**	6	*<0.01*	**0.65**	**2.83**	-
Tumor: HT29 colon carcinoma										
57	1	**2.3**	54	*<0.01*	**5.0**	64	*0.10*	**8.2**	**12.2**	-
Vincristine	1	**3.1**	73	*0.02*	**4.5**	61	*0.04*	**8.3**	**10.9**	-

Treatments on days 1, 5, 9 by intravenous administration (except vincristine given as intraperitoneal injection). Since data are derived from multiple studies, tumor size measurements were made on one of the days indicated in the day range. Survival was 100% for all mice at the indicated doses during the study. RTG = relative tumor growth compared to day 0; %T/C = relative tumor growth of treated vs. control (vehicle) group; P-value = Student's t-test significance.

Example 57 inhibits the growth of a human tumor cell line that is resistant to a standard chemotherapeutic agent

The activity of Example 57 was studied against tumors derived from cell lines that have inherent resistance to vincristine, paclitaxel, or docetaxel. Example 57 at a doses below the maximally tolerated dose of 1.5 mg/kg inhibited growth 83-97% compared to vehicle-treatment in DLD1 colon carcinoma (Figure 1), as well as in HCT-15 and SW620W colon carcinoma models (data not shown; Figure 1 is representative of these studies). Minimal response was obtained with vincristine near its maximally tolerated dose of 1 mg/kg. These tumors also have previously been observed to be poorly responsive to paclitaxel or docetaxel. Resistance in these tumors is likely mediated by multidrug resistance protein (P-glycoprotein, MDR1), but other mechanisms may also exist. Example 57 inhibits growth of these tumor models at doses similar to Example 129. Slightly higher doses of these agents are sometimes needed to achieve efficacy in these paclitaxel- and vincristine-resistant tumors compared to other tumor models, although these doses are still 2-4 fold below their maximally tolerated doses. These data suggest that patients with paclitaxel- or vinca-resistant tumors may respond to Example 57.

Figure 1: Effect of Example 57 on vincristine-resistant human tumor xenografts in athymic mice.



Groups of 5 female athymic mice were injected with DLD1 cells. Animals bearing staged tumors were treated on days 1, 5, and 9 with saline vehicle (IV), Example 57 (1 mg/kg IV), Example 129 (1 mg/kg IV), or vincristine (1 mg/kg IP). Data are mean fold increase in tumor volume in each group.